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(54) Title: A METHOD FOR SENSITIZATION OF CANCER CELLS FOR KILLER CELL MEDIATED LYSIS			
(57) Abstract <p>The invention relates to a method of sensitizing cancer cells for a killer cell mediated lysis which involves administering to a patient an effective amount of antiestrogen and killer cells either jointly or sequentially, wherein the killer cells are selected from the group of NK cells, LAK cells and CTL cells and the antiestrogen is selected from the group of triphenylethylene class antiestrogens, such as tamoxifen or toremifene or their pharmaceutically acceptable salt.</p>			

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<p>(54) Title: A METHOD FOR SENSITIZATION OF CANCER CELLS FOR KILLER CELL MEDIATED LYSIS (57) Abstract The invention relates to a method of sensitizing cancer cells for a killer cell mediated lysis which involves administering to a patient an effective amount of antiestrogen and killer cells either jointly or sequentially, wherein the killer cells are selected from the group of NK cells, LAK cells and CTL cells and the antiestrogen is selected from the group of triphenylethylene class antiestrogens, such as tamoxifen or toremifene or their pharmaceutically acceptable salt.</p>		

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A METHOD FOR SENSITIZATION OF CANCER CELLS FOR KILLER CELL MEDIATED LYSIS

BACKGROUND OF THE INVENTION

The invention relates to the treatment of cancer by sensitization of
5 cancer cells by antiestrogens for lysis with killer cells, particularly with natural
killer (NK) cells, lymphokine activated killer (LAK) cells and cytotoxic T
lymphocytes (CTL).

The treatment of human cancer with autologous lymphokine activated
killer (LAK) cells combined with recombinant-derived lymphokine, interleukin-
10 2, has already been attempted with encouraging results (Rosenberg, 1987)
as well as the treatment with activated cytotoxic T lymphocytes (CTL)
(Fujimoto, 1992).

Nonsteroidal antiestrogens tamoxifen and toremifene belonging to the
triphenylethylene class of compounds have gained wide therapeutic
15 application for the treatment of estrogen receptor positive breast cancer.
Tamoxifen and toremifene inhibit estrogen-induced growth by competitive
antagonism of tumor estrogen receptors. Antiestrogen therapy is effective in
prolonging a disease-free state and overall survival of women following
primary surgery. Other well known triphenylethylene class antiestrogens are
20 e.g. clomiphene and droloxifene (3-hydroxytamoxifen).

We have now discovered that estrogen receptor negative cancer cells
are sensitized by triphenylethylene antiestrogens for lysis with NK, LAK and
CTL effectors. This sensitization effect is thus not dependent on the presence
of classical estrogen receptors. The use of the combination of antiestrogen
25 treatment and killer cell therapy according to the invention is expected to
significantly elevate the percentage of tumor remission and cure that has
already been achieved by above investigators with killer cells alone in a minor
percentage of patients.

SUMMARY OF THE INVENTION

30 The invention relates to a method of sensitizing cancer cells for a killer
cell mediated lysis which involves administering to a patient an effective

amount of antiestrogen and killer cells either jointly or sequentially, wherein the killer cells are selected from the group of NK cells, LAK cells and CTL cells and the antiestrogen is selected from the group of triphenylethylene class antiestrogens, such as tamoxifen or toremifene or their pharmaceutically acceptable salt. Alternatively, killer cells may be induced in a host by one of known immunostimulation methods during the first treatment period and thereafter administering an effective amount of antiestrogen during the second treatment period.

BRIEF DESCRIPTION OF THE FIGURES

- 10 FIG. 1 is a graph of a Winn assay with untreated, tamoxifen (TX) treated and toremifene (TO) treated P815 tumor cells.
- FIG. 2 a, b and c are the survival curves for the experiment of Fig. 1.
- FIG. 3 is a graph of the effect of oral TX and TO treatment alone or with LAK treatment on P815 tumor growth.
- 15 FIG. 4 a, b and c are the survival curves for the experiment of Fig. 3.
- FIG. 5 is a graph of a winn assay with untreated, tamoxifen (TX) treated and toremifene (TO) treated P815 tumor cells.
- FIG. 6 a, b and c are the survival curves for the experiment of Fig. 5.
- FIG. 7 is a graph demonstrating the immunotherapy results of P815
- 20 mastocytoma with TX or TO and LAK cells.
- FIG. 8 a, b and c are the survival curves for the experiment of Fig. 7.
- FIG. 9 is a graph demonstrating the immunotherapy results of P815 mastocytoma with TX or TO given by gavage and LAK cells.
- FIG. 10 a, b and c are the survival curves for the experiment of Fig. 9.

25

DETAILED DESCRIPTION OF THE INVENTION

1. Detection of killer cells

The prerequisite for success of the treatment according to the invention is the presence of killer cells (e.g. NK, LAK or CTL) capable of destroying the patient's cancer cells in the body when the adjuvant therapy with an antiestrogen, e.g. tamoxifen (TX) or toremifene (TO), is initiated. Ideally, a suspension of cells would be prepared from the patient's tumor by digestion with collagenase as described by Freshney (1976) and used as target cells. Some of the targets will then be pretreated for 4 hours with either TX or TO

while others are left untreated, and both preparations are labeled with ^{51}Cr . Patient's peripheral blood mononuclear cells depleted of adherent cells are used as effectors in ^{51}Cr release assay. Surplus tumor cells may be stored in tissue culture medium, such as RPMI-1640, supplemented with 10% serum
5 (preferably autologous) and 10% dimethylsulphoxide in liquid nitrogen for later use. If this approach is not feasible, one may be able to use cell lines as targets, such as K652, which is suitable for the detection of NK activity, or lines derived from the same type of cancer the patient is suffering from. The presence of tumor infiltrating lymphocytes (TIL) in biopsy specimen may be
10 taken as indication for the presence of host derived killer cells. Additional procedures that are used to detect cell mediated immunity and could be of use are: delayed type hypersensitivity skin reactions elicited by tumor antigen/extract; inhibition of peripheral leukocyte migration by tumor antigen/extract; and the proliferative response of CD8+ T lymphocytes to tumor antigen/extract
15 in vitro.

Once it has been established by any one of the above procedures that the patient harbours killer cells capable of killing his/her cancer, antiestrogen therapy will be initiated until tumor regression is achieved. If killer cells are not present they should be induced in a host by reduction of tumor burden or by
20 immunostimulation methods as described below.

2. Reduction of tumor burden

Most patients with overwhelming tumor burden will not have detectable killer cells in their circulation. Here the first task would be to reduce tumor burden, if possible, by surgery, radiation or chemotherapy. There are
25 numerous animal experiments and observations on patients to indicate that the reduction of tumor burden frequently leads to the appearance of killer cells, both in the circulation and in the tumor itself. Once the killer cells are detectable, the adjuvant therapy with the antiestrogen would commence.

3. Immunostimulation

30 If the reduction of tumor burden does not lead to the spontaneous appearance of killer cells in the patient, the stimulation of such killer cells may be attempted. Some of the approaches that can be used are listed below:

(a) Vaccination.

35 (b) Treatment with cyclophosphamide, which is known to inhibit suppressor immune mechanisms, and thus to enhance cellular

immunity. Similarly, indomethacin interferes with the suppressive effect of macrophages.

(c) By the application of cytokines, such as interleukin-2.

(d) By the use of adjuvants stimulating cell mediated immunity such as
5 muramyl peptide and synthetic bacterial wall analogues (Utsugi et al, 1991).

(e) By dietary factors, such as vitamin A (Gergely et al, 1988).

Suitable vaccination procedures are described by Livingston, 1992; Chang et al, 1993 and Hoover et al, 1993. Cyclophosphamide treatment is described, for example, by Komatsumoto et al, 1991 and Kassabov and
10 Stoychkov, 1991. Indomethacin treatment is described, for example, by Saarloos et al, 1993 and Aso et al, 1992.

The usual therapeutic dose for cyclophosphamide is 50 mg daily and for indomethacin 50 mg daily. Cytokines, such as interleukin-2 (IL-2), may also be used for immunostimulation as described e.g. in Yoshino et al. (1991).
15 Yoshino et al. treated patients with low dose IL-2 (2×10^6 Japan reference units s.c. initially, which dose was decreased to half on each consecutive day for 6 days) which resulted in significant activation of NK and LAK cells.

Once killer cells have been induced, target sensitization with antiestrogen therapy would commence.

20 4. Joint therapy with killer cells

Administering in vitro activated killer cells of NK, LAK or CTL type and antiestrogen either jointly or sequentially is within the scope of the invention. Antiestrogen is selected from the group of triphenylethylene antiestrogens. The preferred antiestrogen is toremifene or tamoxifen or their pharmaceutical-
25 ly acceptable salt. Our experiments show that such combination therapy has a definite advantage in comparison with either drug treatment or killer cell therapy alone. According to our experiments target cell sensitization is optimally exploited at the stage when killer cells are already generated by the patient, or alternatively, when the killer cells are applied jointly with the drugs.

30 For the generation of LAK cells, patient's mononuclear lymphocytes are cultured with interleukin-2. The mononuclear lymphocytes are collected as described in Rosenberg et al (1987). The procedure can be used in order to collect about 1×10^8 to 1×10^{11} mononuclear cells per patient. The lymphocytes can then be separated from the adherent cells using known
35 procedures. The resulting mononuclear lymphocytes are cultured in any

appropriate media such as RPMI-1640 supplemented with human recombinant interleukin-2, for example at 37°C for three to five days. The resulting LAK cells are isolated and suspended in media suitable for intravenous infusion into the patient. The LAK cells are administered preferably once every day or every other day for from one to five daily doses. Preferably from 1×10^8 to 1×10^{10} LAK cells are administered daily on each of three days over a period of from 3 to 10 days. The patient may require from one to four such regimens sequentially. NK and CTL cells may be administered in a similar fashion. Further guidelines for NK and CTL therapy may be obtained from the publications of Rosenberg et al. (1987) and Fujimoto (1992).

The doses for TX and TO and treatment schedule that are currently in use would be applied in therapy according to the invention. For TX an suitable oral dose is 20 - 40 mg given daily (Anderson et al., 1981). The suitable oral dose for TO is 40 - 60 mg, but can be as high as 240 mg daily (Hietanen et al., 1990). Preferably the antiestrogen treatment is started as soon as the killer cells are generated or infused into the patient. The antiestrogen therapy and killer cell therapy are then maintained jointly, optimally until the complete cure of the patient. It is also within the scope of the invention to apply the antiestrogen therapy and killer cell therapy sequentially, i.e. killer cell therapy is commenced immediately after discontinuation of antiestrogen therapy. However, the joint therapy is the preferred procedure.

In conclusion, the treatment procedure of using TX or TO to enhance cell mediated host defense according to the invention is preferably as follows:

- (a) Tests are performed for the detection of killer cells in the patient;
- (b) if killer cells are not present they are induced by known methods, or
- (c) killer cells are generated in vitro and infused to the patient;
- (d) TX or TO adjuvant therapy is applied as soon as the killer cells are generated or infused into the patient, preferably after the determination of the sensitizing effect of these drugs on the target cell in question for lysis by the effector cells available;
- (e) during therapy with the sensitizing drugs the patient is followed closely for effectiveness.

Various modifications can be made in the therapeutic method of the present invention without departing from the spirit and scope thereof. The

embodiments described herein are for the purpose of illustrating the invention but are not intended to limit it.

Example 1.

The effect of toremifene (TO) and tamoxifen (TX) on target cell lysis by natural killer (NK) cells, lymphokine activated killer (LAK) cells, and cytotoxic T lymphocytes (CTL) was studied. Rat spleen cells were used as effector cells either without activation (NK cells), or after treatment with human IL-2 (LAK), or after stimulation with the Nb2 rat lymphoma in mixed cultures (CTL). The Yac-1 murine lymphoma was used as a target for NK cells, the P815 mastocytoma for LAK cells and Nb2 cells for CTL. Target and effector cells were pretreated with TO or TX, at concentrations from 1 nM to 5 μ M, for 4 hours and then washed. Target cells were labeled with ^{51}Cr for 1 hr, then washed. The specific ^{51}Cr release was determined after 4 hours of incubation at 37 °C, 5 % CO_2 (target/effector 1:25). The results presented in Tables 1-3 show that TO and TX enhanced target cell lysis in a dose dependent fashion with NK, LAK or CTL effectors, if the target cells were treated for 4 h prior to the cytotoxic reaction. The pretreatment of effector cells had no such enhancing effect, but TO or TX treated effectors lysed treated targets as efficiently as did nontreated effectors.

TABLE 1. The effect of tamoxifen (TX) and toremifene (TO) on NK cell mediated cytotoxicity .

n	Treatment	Percent specific ^{51}Cr release		
		Target treated	Effector treated	Both treated
25	7 none	10 \pm 1	10 \pm 1	10 \pm 1
	4 TX 1 μ M	74 \pm 2	1 \pm 0.2	77 \pm 2
	4 TX 100 nM	68 \pm 1	0.3 \pm 0	74 \pm 2
	2 TX 10 nM	61 \pm 1	0 \pm 0	62 \pm 1
	2 TX 1 nM	30 \pm 2	0 \pm 0	42 \pm 2
30	4 TO 5 μ M	65 \pm 3	1.3 \pm 0.7	68 \pm 3
	4 TO 1 μ M	54 \pm 3	1.0 \pm 0.4	59 \pm 3
	4 TO 100 nM	48 \pm 2	0.3 \pm 0	52 \pm 1
	3 TO 10 nM	40 \pm 1	0 \pm 0	41 \pm 2
	2 TO 1 nM	25 \pm 3	0 \pm 0	30 \pm 2

Target cells: Yac-1 murine lymphoma

Effector cells: Spleen lymphocytes of female Fischer rats

TABLE 2. The effect of tamoxifen (TX) and toremifene (TO) on target cell destruction by cytolytic lymphocytes generated in mixed cultures.

5	n	Treatment	Percent specific ^{51}Cr release		
			Target treated	Effector treated	Both treated
	2	none	11 ± 1	11 ± 1	11 ± 1
	2	TX $1 \mu\text{M}$	76 ± 2	8 ± 0	82 ± 2
	2	TX 100 nM	45 ± 2	5 ± 1	52.5 ± 1.5
10	2	TO $5 \mu\text{M}$	62 ± 2	5.5 ± 0.5	61 ± 1
	2	TO $1 \mu\text{M}$	52 ± 2	3.5 ± 0.5	54 ± 2
	2	TO 100 nM	31 ± 1	1.5 ± 0.5	35 ± 3
	2	TO 10 nM	25 ± 1	0 ± 0	22 ± 2
	2	TO 1 nM	16 ± 1.5	0 ± 0	14.5 ± 3.5

15

Target cells: Nb2 rat lymphoma

Effector cells: Cytotoxic T- lymphocytes (CTL) generated in mixed cultures, harvested on day 5

Responder: Spleen cells of Fischer rats. Stimulator: Nb2 cells (thymic lymphoma of Noble rats).

20 TABLE 3. The effect of tamoxifen (TX) and toremifene (TO) on LAK cell mediated cytotoxicity .

25	n	Treatment	Percent specific ^{51}Cr release		
			Target treated	Effector treated	Both treated
	3	none	10 ± 2	10 ± 2	10 ± 2
	2	TX $1 \mu\text{M}$	78 ± 0	2 ± 0	84 ± 1
	1	TX 100 nM	72 ± 0	2 ± 0	82 ± 0
	3	TO $5 \mu\text{M}$	75 ± 4	2 ± 0.3	77 ± 2
30	3	TO $1 \mu\text{M}$	66 ± 3	0 ± 0	70 ± 0
	3	TO 100 nM	59 ± 1	0 ± 0	61 ± 1
	3	TO 10 nM	48 ± 1	0 ± 0	50 ± 0.3
	3	TO 1 nM	36 ± 2	0 ± 0	43 ± 2

35

Target cells: P815 (NK-resistant murine mastocytoma)

Effector cells: Spleen cells of Fischer rats were cultured in medium supplemented with 500 U/ml rIL-2 for 6 days at 37°C and $5\% \text{ CO}_2$.

Example 2.

For the generation of LAK cells, 2×10^6 /ml of spleen cells from female DBA/2 mice were cultured for 5 days in RPMI-1640 medium supplemented with 10%FCS, 5×10^{-5} M 2-mercaptoethanol and 500 U/ml of human recombinant Interleukin-2 at 37°C in humidified air containing 5% CO₂. The LAK cells generated killed, at 1:25 ratios, 10 % of P815 cells, 52 % of tamoxifen (TX) treated (1 μ M, 4 h) and 51 % of toremifene (TO) treated (5 μ M, 4 h) cells in the ⁵¹Cr assay. The same effector cells were then mixed with the same untreated or drug treated P815 cells at 1:25 target:effector cell ratios and injected s.c. to syngenic DBA/2 recipients (Winn assay). Groups of 4 female animals were used and tumor growth was followed until all controls succumbed to neoplasia. Further details of the experimental design and tumor size are presented in Figure 1. In this figure only those animals that developed a tumor are included. The survival curves are presented in Figures 2 a, b and c.

As is obvious from the results, drug treatment alone or LAK cells alone exerted some tumor inhibition, and when both drug treatment and effector cells were applied, tumor inhibition was enhanced. For instance, tumor cells exposed to TX or LAK cells killed all the animals, whereas one animal was protected in the TX + LAK group. Pretreatment with TO protected 1 of 4 animals, whereas TO + LAK protected 2 of 4 animals. Also, it is clear from Fig. 1 that there is a marked retardation of tumor growth in the drug + LAK treated groups between days 17-23.

Example 3.

In the previous experiment there was only a brief exposure of the tumor cells to the drug (4 h) and to LAK cells, which explains that tumor retardation was limited to the early period in those animals which eventually grew the tumor. In the next experiment the same basic design was used, but this time drug treatment was given in the drinking water (TX, 100 μ g/ml and TO, 400 μ g/ml) starting 2 days prior to tumor injection and maintained throughout the experiment. Again, some of the groups received tumor cells mixed with LAK cells at 1:25 ratio and treated further with the same dose of LAK cells injected to the tumor site weekly for 3 weeks. This time significant tumor inhibition occurred in the animals treated with either drug alone and also in those receiving LAK cells only, as can be seen Figure 3. Again, tumor growth was

the slowest in those groups with both drug and LAK treatment. Moreover, there is one animal in the TX + LAK group and also in the TO and TO + LAK group which is tumor free at this time, as can be seen in Figure 4 a, b and c. Therefore, with continuous treatment we succeeded in slowing tumor growth
5 considerably although the proportion of tumor free animals did not increase.

Example 4.

The experiment of Example 2 was repeated with increased amount of LAK cells (1:50 ratio) mixed with drug treated or nontreated tumor cells and applied s.c. to animals. The results are shown in Figure 5. The survival curves
10 for Fig. 5 are shown in Figure 6a, b and c. Again, as in previous experiments, there are some tumor free animals in the treated groups, especially when drug treatment is combined with killer cells.

Example 5.

In this immunotherapy experiment all groups received the same dose of
15 tumor cells s.c. and 12 days later when the tumor was clearly palpable in all animals, drug treatment in the drinking water was initiated. Treatment with LAK cells was started 2 days later and given 3 times, 4 days apart, as indicated in Figure 7. Drug treatment was maintained throughout the experiment. As is obvious from Fig. 7, tumor growth was markedly retarded by
20 drug treatment combined with killer cells. This is also reflected in the survival curves, which are presented in Figure 8a, b and c. All the controls succumbed to neoplasia by day 26, whereas all the animals are still alive on day 28 in the TO + LAK group. There is one total regression in TX + LAK and one partial regression in TO + LAK treated groups (the tumor volume decreased to 10
25 times less than the original volume).

Example 6.

DBA/2 female mice were injected s.c. with 10^4 P815 tumor cells. When the tumors were approximately 0.5 cm diameter, drug treatment was introduced (either TX or TO, 10 mg/kg/day by gavage). Two days later 25×10^4
30 LAK cells were given i.p., LAK was injected three times more, four days apart. Drug treatment was maintained from day 14 until day 30. The results are shown in Fig. 9. Again, tumor growth was markedly retarded by drug treatment combined with killer cells. The survival curves are presented in Fig. 10 a, b and c. There was one total regression in TX + LAK and one partial
35 regression in TO + LAK treated group (the tumor volume decreased to 10

times less than the original volume). Furthermore there was one partial regression both in TX group and TO group.

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CLAIMS

1. A method of sensitizing cancer cells for a killer cell mediated lysis which comprises administering to a host in need thereof an effective amount of
5 antiestrogen and killer cells either jointly or sequentially, wherein the killer cells are selected from the group of NK cells, LAK cells and CTL cells and the antiestrogen is selected from the group of triphenylethylene antiestrogens.

2. A method of claim 1 in which the triphenylethylene antiestrogen is tamoxifen or toremifene or their pharmaceutically acceptable salt.

10 3. A method of claim 2 in which tamoxifen or its pharmaceutically acceptable salt is orally administered in a daily amount of 20 - 40 mg.

4. A method of claim 2 in which toremifene or its pharmaceutically acceptable salt is orally administered in a daily amount of 40 - 240 mg.

5. A method of sensitizing cancer cells for a killer cell mediated lysis which
15 comprises inducing killer cells to a host by one of known immunostimulation methods during the first treatment period and thereafter administering an effective amount of a triphenylethylene antiestrogen during the second treatment period.

6. A method of claim 5 in which the triphenylethylene antiestrogen is
20 tamoxifen or toremifene or their pharmaceutically acceptable salt.

7. A method of claim 5 or 6 in which the immunostimulation is accomplished by administration of interleukin-2.

8. Use of a triphenylethylene class antiestrogen in the manufacture of a medicament for sensitizing cancer cells for a killer cell mediated lysis.

25 9. Use as in claim 8 in conjunction with killer cells selected from the group of NK cells, LAK cells and CTL cells.

10. Use as in claim 8 in conjunction with an immunostimulating agent such as interleukin-2.

11. A triphenylethylene class antiestrogen for use in conjunctive systemic
30 administration with killer cells in sensitizing cancer cells for a killer cell mediated lysis.

1 / 10

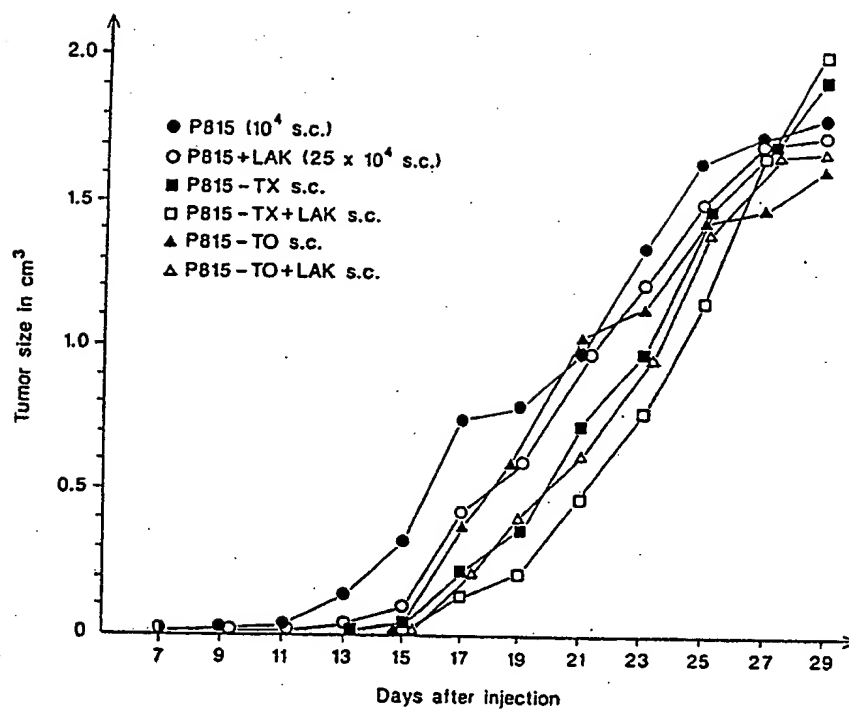
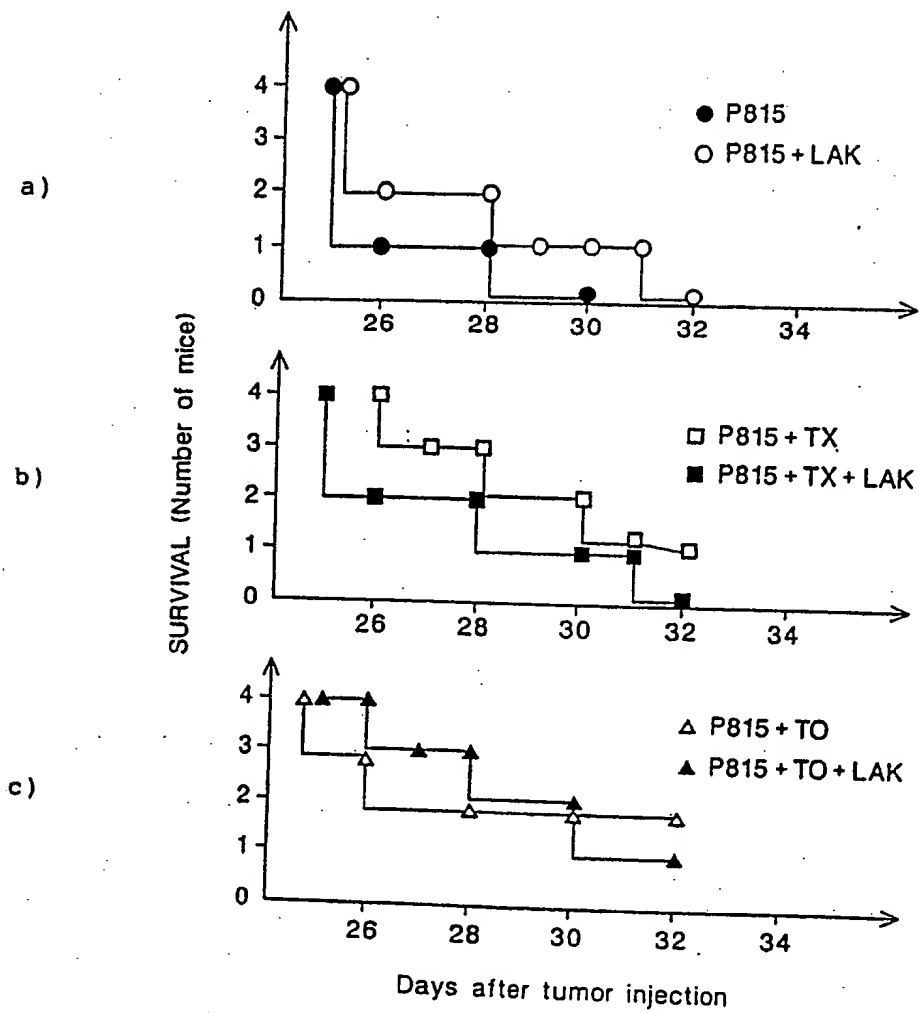


Fig. 1

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3 / 10

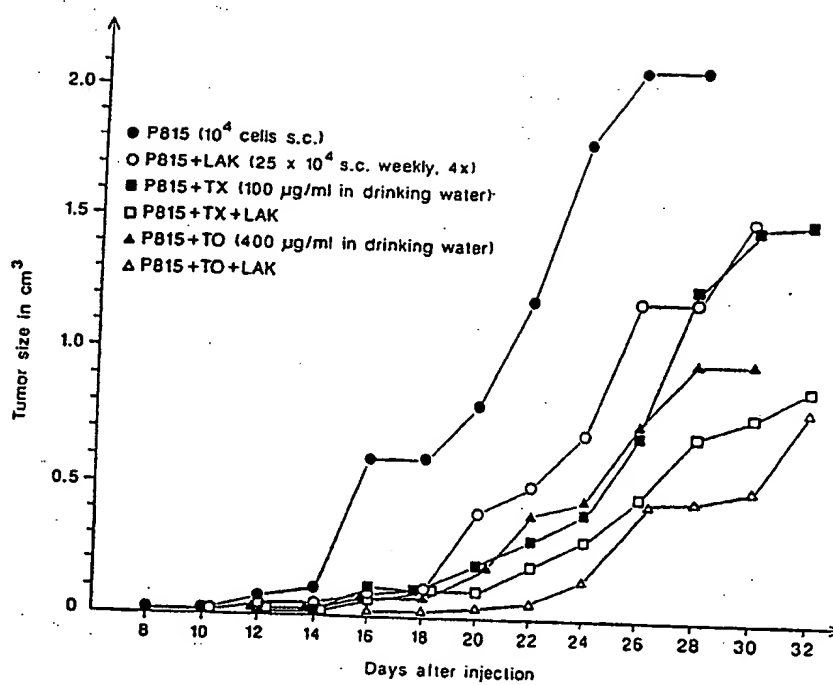


FIG. 3

4 / 10

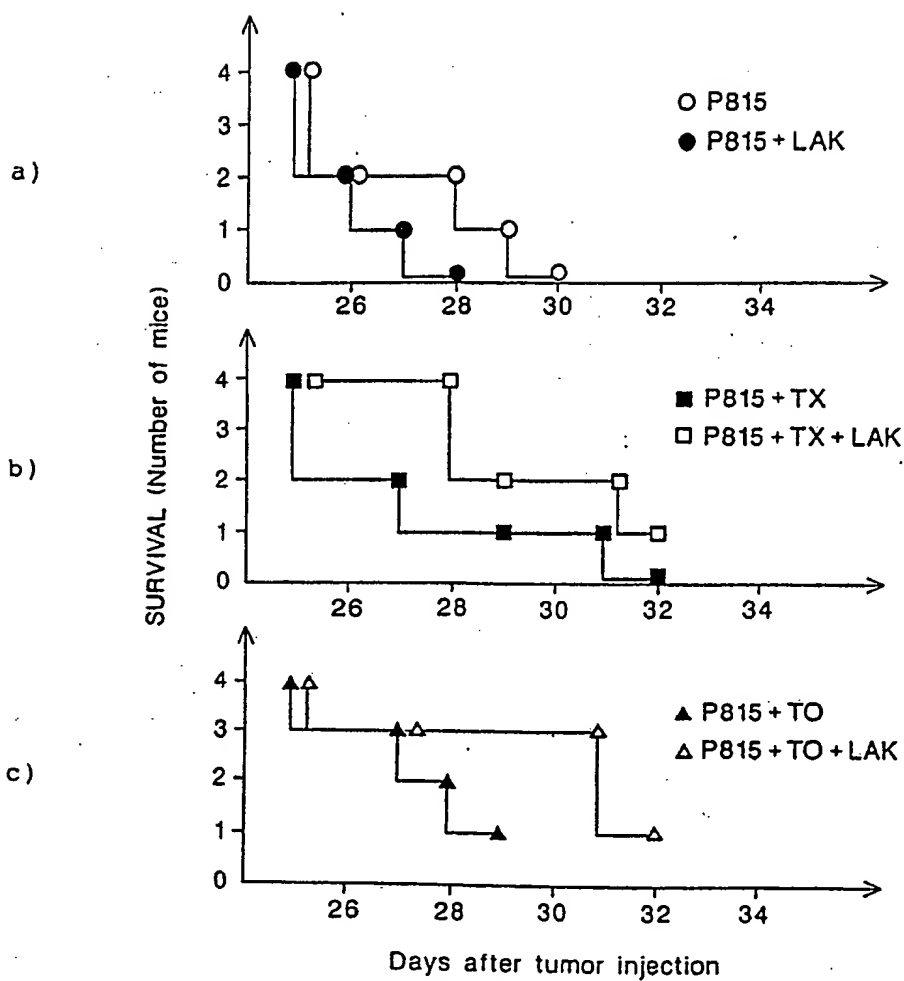


FIG. 4

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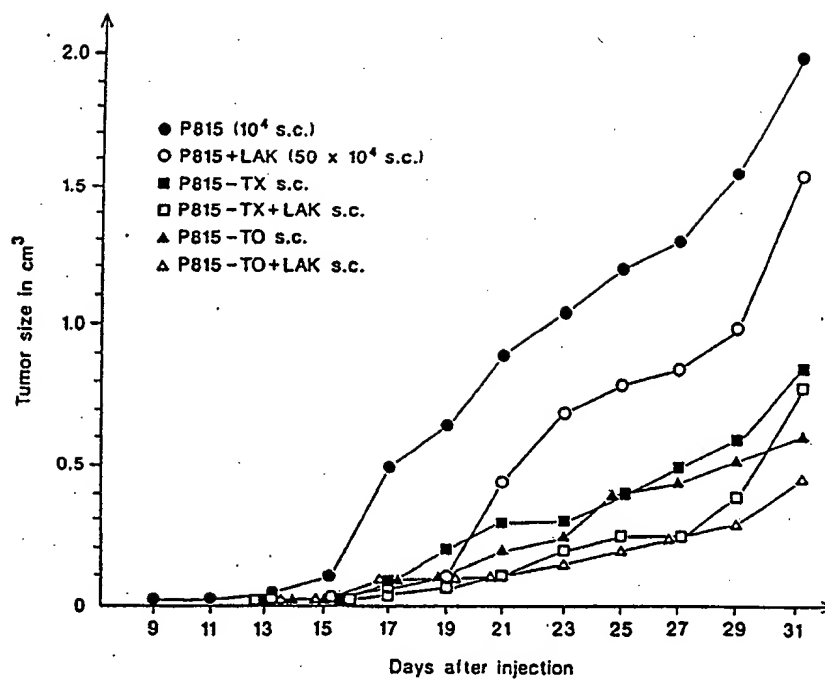


Fig. 5

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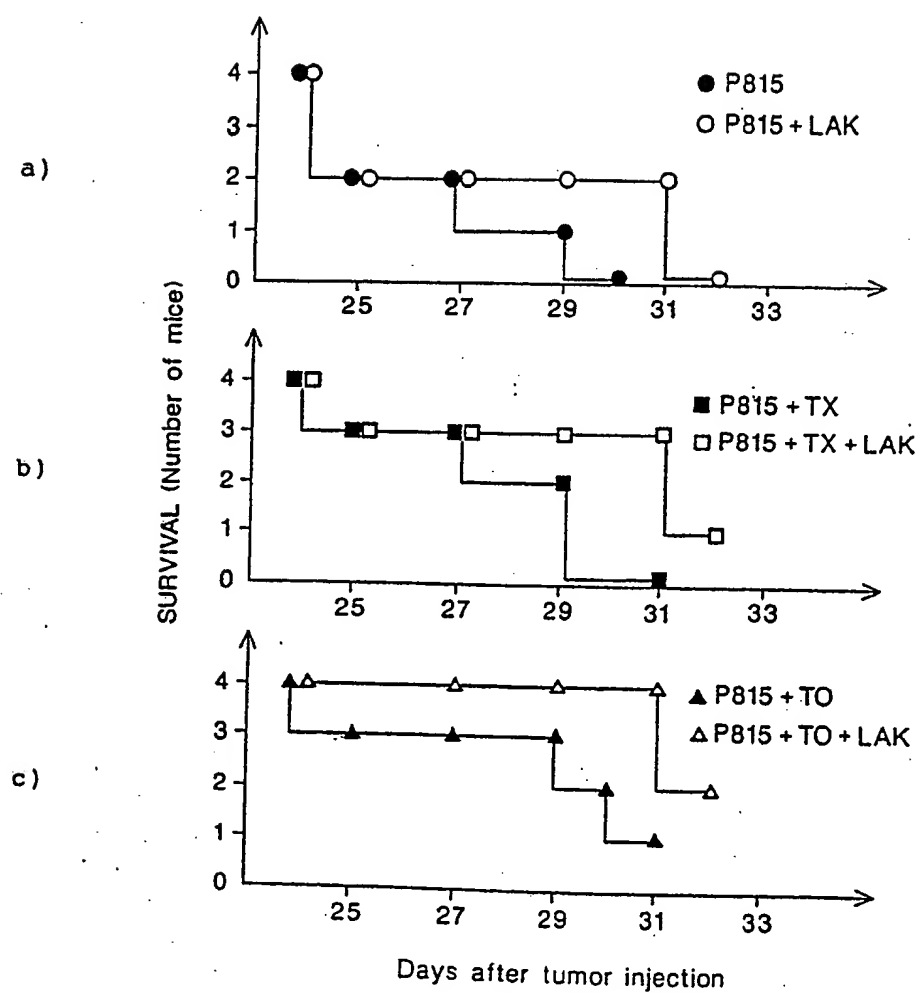


Fig. 6

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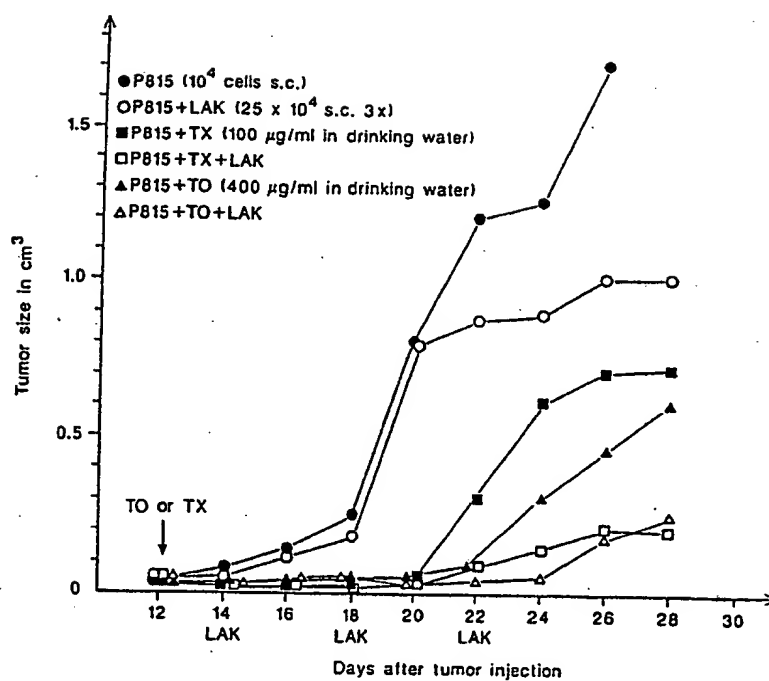


Fig. 7

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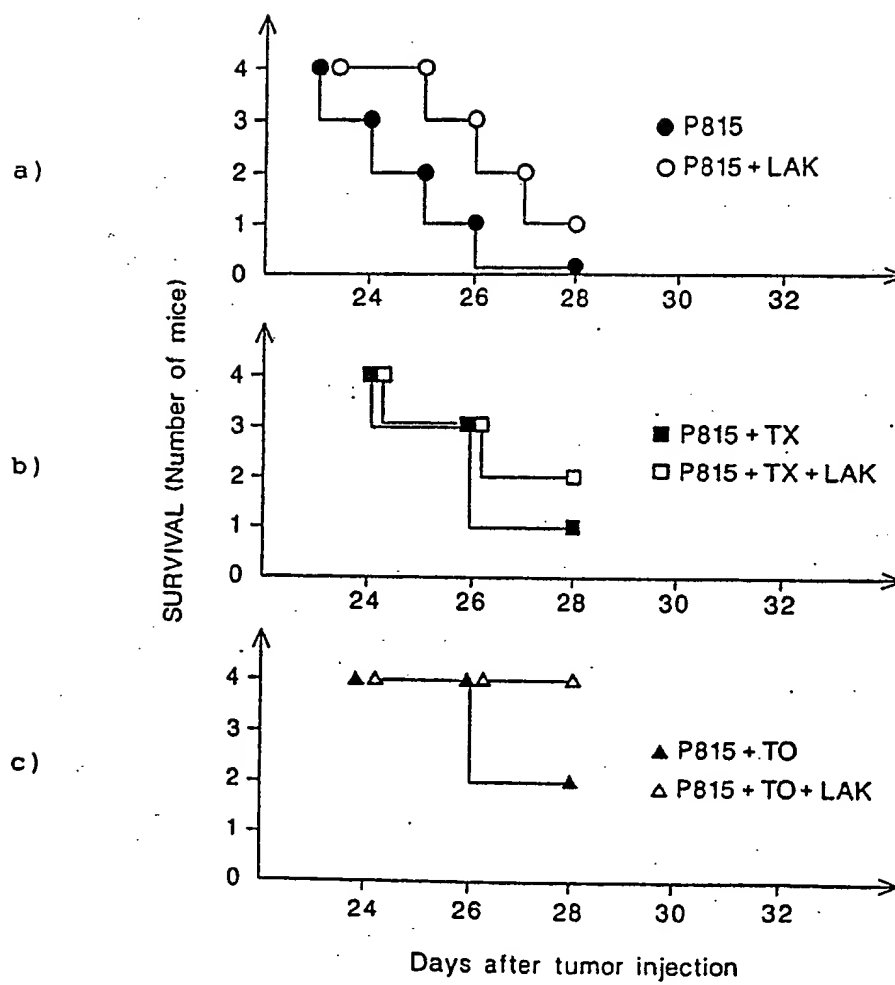


Fig. 8

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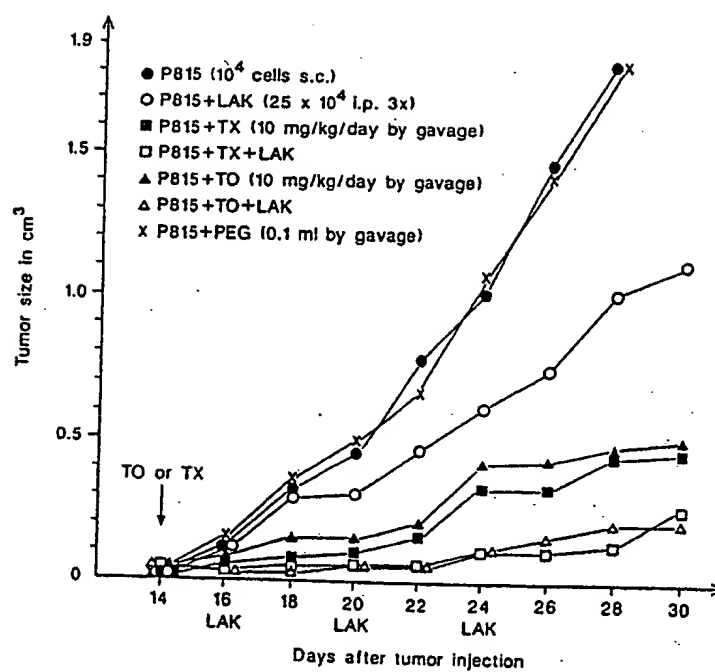


Fig. 9

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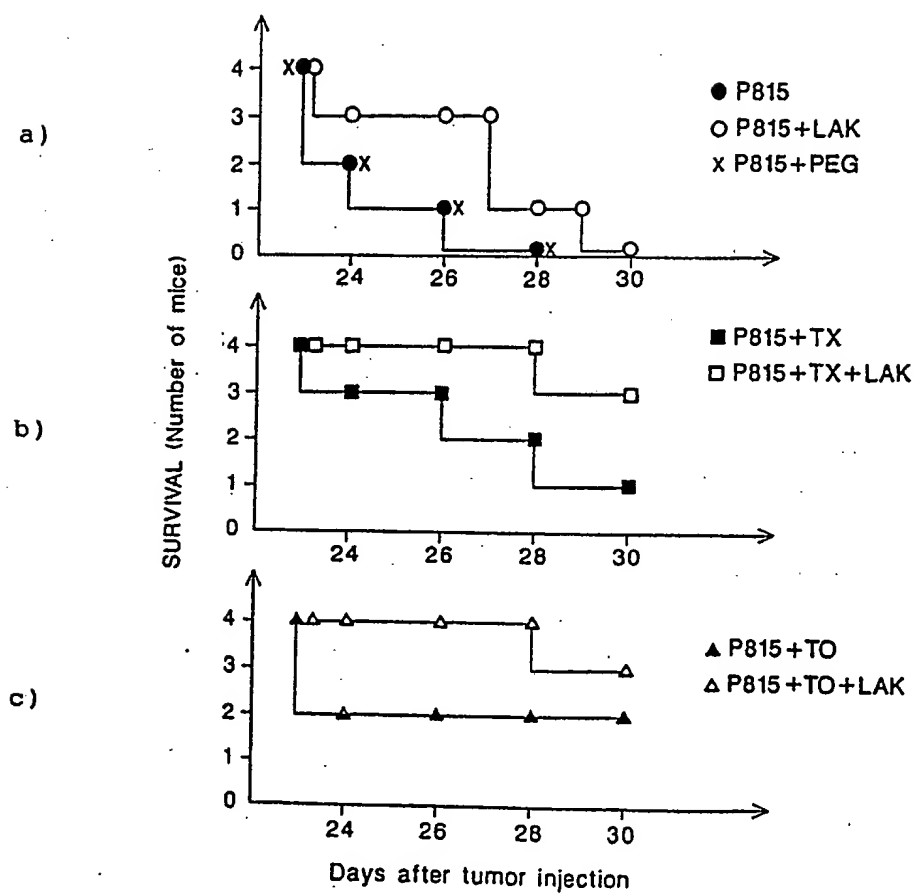


Fig. 10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 94/00333

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 38/22, A 61 K 35/78, A 61 K 35/55, A 61 K 35/14,
A 61 K 31/01

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5 057 423 (HISERODT et al.) 15 October 1991 (15.10.91), claims 1,2; column 1, lines 11-37. ---	1,5, 7-11
Y,P	EP, A, 0 589 039 (ASAHI KASEI KOGYO KABUSHIKI KAISHA) 30 March 1994 (30.03.94), claims 1,3; page 3, lines 8-13. ----	1,2, 5,6, 8,11

☐ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search
07 November 1994

Date of mailing of the international search report

29 -11- 1994

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BÖHM e.h.

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/FI 94/00333 SAE 94531

In diesem Anhang sind die Mitglieder
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In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 5057423	15-10-91	FR A1 2624742 JP A2 2109976	23-06-89 23-04-90
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